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EDITORIAL REVIEW

Thromboxane synthesis and action within the kidney

Thromboxane (Tx) A_2 is a labile derivative of arachidonic acid metabolism that amplifies the platelet response to a variety of aggregating agents [1]. In addition, TxA_2 contracts vascular smooth muscle [2] and glomerular mesangial cells [3] through receptor-mediated actions, resulting in increased intracellular calcium [4–6]. TxA_2 is formed by the sequential cyclooxygenation and peroxidation of arachidonic acid to the prostaglandin (PG) endoperoxides, PGG_2 and PGH_2. The last of these compounds is then transformed to TxA_2 by a specific TxA_2 synthase.

Platelets represent a major source of TxA_2 formed in vivo under physiological conditions. However, TxA_2 synthase is widely distributed in other cells, particularly in macrophages, in the lung, peritoneum and kidney [7, 8], which may augment their contribution to systemic TxA_2 biosynthesis in disease states. The most convincing evidence for the biological importance of TxA_2 and its precursor endoperoxides derives from the beneficial effects of aspirin in platelet-mediated vascular occlusive disease [9]. Aspirin irreversibly inhibits platelet cyclooxygenase activity by acetylating a serine residue of the enzyme PGG/H synthase [10, 11]. This mechanism is presumed to underlie its effectiveness in preventing death in unstable angina [12–14] and in patients who have suffered a myocardial infarction [15], conditions in which TxA_2 biosynthesis is augmented [16].

More recently, evidence has accumulated to support a role for these eicosanoids in the pathogenesis of various forms of renal disease. The purpose of this review will be to consider the role of TxA_2 within the kidney and to consider the potential therapeutic benefit which might derive from modulating TxA_2 synthesis and/or action by pharmacological or dietary intervention.

Mechanism of action of TxA_2

Due to the evanescence of TxA_2, which has a half-life of 30 seconds at physiological pH [1, 17], the receptor site for this compound has been characterized using structural analogs of PGH_2 and, more recently, TxA_2 itself [18–20]. Indeed, the chemical structure of TxA_2 was at first merely hypothesized and only confirmed 10 years later when a TxA_2 macrolactone was synthesized and the effects on platelets and vascular smooth muscle of the biologically generated material were replicated [17]. Given the limitation of the ligands used to characterize PGH_2/TxA_2 receptor sites, it has been thought that both eicosanoids have a similar affinity for a common receptor site on platelets [21].

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Recent observations suggest that the situation might be more complex. Thus, the antagonist ligand, GR 32191 [22], identifies two sites on human platelets; it dissociates rapidly from one (GR_r) and appears to bind irreversibly (GR_{irr}) to the other [23]. GR_{irr} sites are coupled to phospholipase C and transduce diacylglycerol formation, phosphoinositide turnover, an increase of intracellular calcium and activation of protein kinase C. These events are associated with platelet activation and secretion. GR_r sites, by contrast, mediate PGH_2/TxA_2-evoked platelet shape change and a major portion of the ligand-induced increase in intracellular calcium, presumably from an IP_3-insensitive source [23]. Discrimination of the shape change and aggregation responses was also achieved with the reversibly bound agonist ligand, BOP [24], although this observation in isolation might have been explained by varied degrees of receptor occupancy necessary to evoke the aggregation and shape change responses [25]. However, this seems an unlikely explanation, given the marked distinction between the plasma half-life of GR 32191 (6 to 7 hrs) and the time to recovery of the ligand-evoked aggregation response ex vivo, which corresponds to platelet turnover time (8 to 10 days). Thus, the pattern of inactivation of PGH_2/TxA_2 receptors on human platelets is consistent with the existence of distinct subtypes of the receptor. It is unknown whether these sites represent two distinct receptor isoforms or varied degrees of posttranslational modification of a single receptor or if the two sites discriminated by GR 32191 differ in their affinities for PGH_2 and TxA_2 [26].

The sequence of a full length cDNA for a PGH_2/TxA_2 receptor obtained from human placenta has recently been reported [27]. Hydrophobicity plotting of the deduced amino acid sequence suggests that the PGH_2/TxA_2 receptor consists of seven transmembrane spanning domains, characteristic of the G protein-linked receptor superfamily. The identity between this clone and a partial length cDNA from a human megakaryocytic cell line was used to suggest the identity of vascular (that is, placental) and platelet (that is, megakaryocyte-derived) PGH_2/TxA_2 receptors [27]. Further information at the molecular level will be necessary to determine if this hypothesis, which is contrary to much biochemical evidence and precedent with most other receptors, is supported.

Studies of the respective hierarchies for structurally distinct agonist and antagonist ligands in platelets and vascular smooth muscle cells suggest that tissue specific heterogeneity is likely [28–30]. However, others who have performed similar experiments, have disputed this interpretation of their data [31–33]. Recently, additional evidence that supports the existence of tissue specific heterogeneity of PGH_2/TxA_2 receptors has been provided [34]. Thus, dissociation of GR 32191 from vascular smooth muscle cells, derived from both rat aorta and human saphenous vein, identifies only a single site, from which the ligand dissociates rapidly. In addition, we have noted that platelet activating factor (PAF) heterologously down regulates

GR_{irr} but not GR_r sites on human platelets [35]. Consistent with the absence from vascular smooth muscle cells of the subtype that mediates aggregation in platelets, agonist evoked responses in this tissue are not down regulated by PAF [34]. Radiolabeled ligands have identified a single class of high-affinity specific binding sites in glomeruli, but no studies have been reported to date with GR 32191.

PGH_2/TxA_2 analogs activate phospholipase C in mesangial cells in a manner analogous to that observed in platelets. They cause an immediate increment in calcium derived from intracellular stores followed by a sustained elevation for longer than 10 minutes, which is dependent upon extracellular calcium. These effects are blocked by a PGH_2/TxA_2 antagonist. Studying this action of the PGH_2/TxA_2 mimetic, U46619 and PGI_2 analog, ilprost, on proliferation of cultured human mesangial cells in the presence or absence of growth factors, Mene et al found multiple functional interactions between eicosanoids and growth factors in the control of mesangial cell proliferation [36]. In particular, U46619 stimulates proliferation when used alone but inhibits proliferation when applied concomitantly with growth factors.

The biochemical mechanisms which regulate the response to PGH_2/TxA_2 have been most clearly defined in platelets. Homologous desensitization of the aggregation response to PGH_2/TxA_2 agonists occurs rapidly, with a half-time of two to three minutes. This results in uncoupling of the aggregation receptor from its phospholipase C-linked G protein [4], with subsequent loss of receptors from the cell membrane. The nature of the G protein involved is unknown, although it is insensitive to pertussis toxin [37], in contrast to that by which thrombin and PAF activate phospholipase C [37–40]. Coincident with these events, the platelet adenylate cyclase is sensitized to stimulation at the post-receptor level [35]. This effect is transduced through the GR_{irr} receptor. PGH_2/TxA_2 released from activated platelets stimulates PGI_2 generation by adjacent endothelial cells [41]. Cross talk between the phospholipase C and adenylate cyclase pathways within thromboxane stimulated platelets would serve to amplify the inhibitory effects of PGI_2. Finally, despite the transduction of these effects via the GR_{irr} receptor and some tenuous evidence that the PGH_2/TxA_2 receptor is phosphorylated upon desensitization [42], neither desensitization of aggregation, nor sensitization of the cyclase, appear dependent on protein kinase C [35].

PGH_2/TxA_2 evoked intracellular calcium responses in human mesangial cells through activation of phospholipase C also undergo rapid, homologous desensitization [6]. In this system, the biochemical mechanisms which underlie this effect and the possible cross talk with other systems remain to be defined. The density of PGH_2/TxA_2 binding sites is reduced in mesangial cells obtained from rats made diabetic with streptozotocin [43], a condition associated with enhanced glomerular TxA_2 synthesis [44].

Evidence for TxA_2 synthesis by resident renal cells

The hypothesis that resident renal cells can metabolize arachidonic acid to prostaglandins and TxA_2 was based on the observation that glomerular capillaries have immunoreactive cyclooxygenase activity [45]. Soon after this observation, two independent laboratories have documented that isolated rat glomeruli convert arachidonic acid into prostaglandins and TxA_2 [46, 47]. Subsequent studies documented that eicosanoid biosynthesis occurs in glomeruli of other species, including humans [48–50]. Similar conclusions were derived from studies on renal epithelial and mesangial cells in culture [51, 52]. PGH_2/TxA_2 evoked intracellular calcium responses in human mesangial cells through activation of phospholipase C also undergo rapid, homologous desensitization [6].

Angiotensin II and arginine-vasopressin stimulate glomerular TxA_2 synthesis via specific receptor(s) linked to phospholipase C [53]. More recently, endothelin has been found to induce the synthesis of TxA_2 by rat and bovine mesangial cells [54, 55]. Indeed studies with antagonists suggest that PGH_2/TxA_2 receptors mediate the contractile response to endothelin in guinea pig tracheal strips and rat aorta [56, 57]. In some [54], but not all [55] systems, the hormonal stimulation of mesangial cell TxA_2 can be blocked by lowering the concentration of extracellular calcium or by incubating the cells with the calcium antagonists, verapamil or nifedipine.

Factors other than hormones which regulate renal TxA_2 biosynthesis include cell-derived mediators, like platelet activating factor (PAF) and interleukin 1 (IL-1), complement membrane attack complex, immune complexes and endotoxin. PAF induces TxA_2 synthesis by rat mesangial cells in culture through a receptor-mediated effect [58]. IL-1 stimulates TxA_2 release after continuous, prolonged exposure to rat mesangial cells [59]. This is suggestive of an effect dependent upon new protein synthesis, perhaps due to regulation of cyclooxygenase message, as has been shown in dermal fibroblasts and endothelial cells [60, 61]. Oxygen-derived free radicals also activate phospholipase A_2, thus enhancing prostaglandin and TxA_2 synthesis in isolated glomeruli through an increase in the availability of arachidonic acid [62]. Cultured mesangial cells exhibit a biphasic response to progressively higher concentrations of hydrogen peroxide. The decrease in eicosanoid formation at higher concentrations is probably due to inactivation of cyclooxygenase [63]. An increase in TxA_2 production occurs in rat glomerular epithelial cells when the terminal complement components C5b-9 are added to the culture medium [64]. *E. coli* lipopolysaccharide stimulates both PGE_2 and TxA_2 synthesis by cultured mesangial cells in a dose- and time-dependent manner [65]. Exposure of mesangial cells to immune complexes [66] or the binding of an antibody to a cell membrane antigen [67] also promotes arachidonic release and eicosanoid formation in mesangial cells.

Another important pathway which may result in renal generation of TxA_2 has been reported by Sraer et al [68] who demonstrated that human glomeruli released a diffusible factor capable of stimulating platelet TxA_2 synthesis. The clinical nature of this factor has not yet been fully identified. GC/MS analysis suggests that at least part of the stimulatory activity is attributable to saturated and monoenoic acids. Thus, injury to glomerular cells may activate platelets by a pathway which is independent from the formation of collagen and leads to local formation of TxA_2

Modulation of renal function by TxA_2 in health and disease

Whole-kidney clearance studies in rats and dogs have shown a consistent fall in glomerular filtration rate (GFR) and in renal plasma flow (RPF) in response to systemic infusion of stable prostaglandin endoperoxide analogues [69–71]. Micropuncture

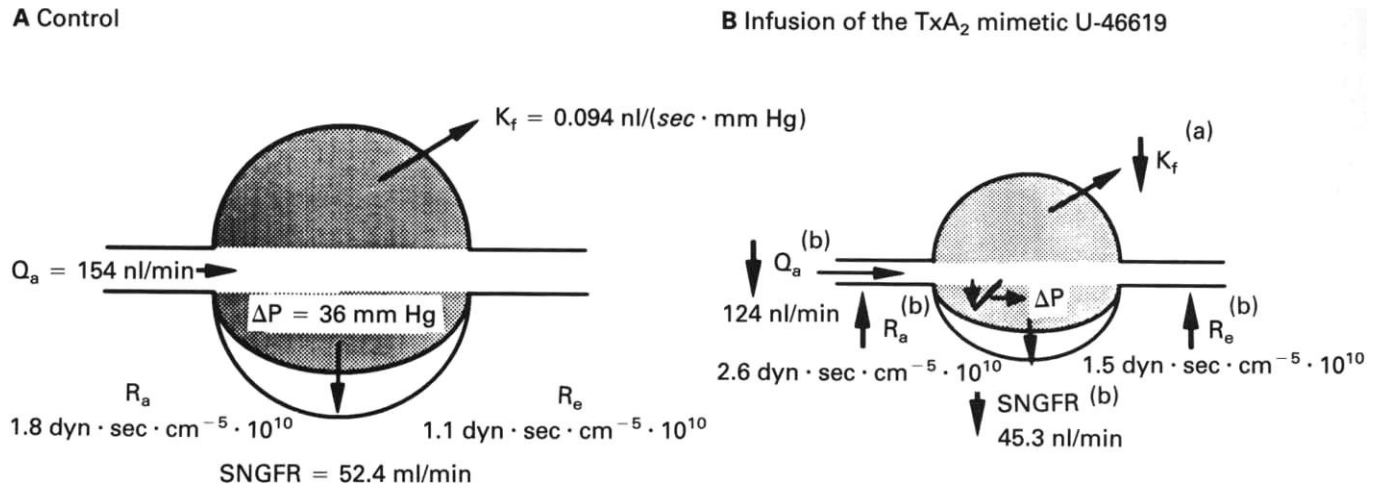

Fig. 1. *Schematic representation of changes in single nephron glomerular filtration rate and its determinants during infusion of the TxA_2 mimetic U-46619 in euvolemic rats.* **(a)**, from reference 76 and **(b)** from reference 69. Abbreviations are: ΔP, mean glomerular transcapillary hydraulic pressure gradient; K_f, glomerular capillary ultrafiltration coefficient; Q_a, glomerular capillary plasma flow rate; R_a and R_e, afferent and efferent arteriolar resistances; SNGFR, single nephron glomerular filtration rate.

studies performed in rats during intraarterial infusion of the PGH_2/TxA_2 analog, U46619, have shown a rise in afferent and efferent arteriolar resistance with a parallel reduction of glomerular capillary flow and single nephron GFR [69] (Fig. 1). Moreover, in an isolated perfused rat kidney preparation, the analog decreased both RPF and GFR. However, the decrease in GFR was more marked than in RPF, suggesting that the mimetic may act selectively upon afferent resistance vessels or may reduce GFR by lowering the ultrafiltration coefficient (K_f) [72].

More recently, this possibility has been addressed directly by examining the effect of PGH_2/TxA_2 analogs on renal microvessels. This has been done in a hydronephrotic perfused kidney model that allows direct visualization of the renal microcirculation [73]. Image analysis of afferent and efferent arteriolar diameters provided direct evidence that PGH_2/TxA_2 preferentially constricts the afferent arteriole. Of interest, is the observation that such a constrictor effect of thromboxane was abolished by calcium antagonists [72]. No changes in K_f were detected since the single nephron GFR and glomerular capillary plasma flow rate (Q_a) fell in parallel. However, most rats in this study were evaluated at filtration pressure equilibrium during infusion of the PGH_2/TxA_2 analog, so that it was not possible to calculate exact values of K_f. Thus, small TxA_2-mediated changes in K_f may have gone undetected.

Consistent with this possibility, GFR falls by a greater extent than RPF in the early phase of nephrotoxic nephritis characterized by enhanced glomerular TxA_2 synthesis, suggesting a fall in K_f [74]. Similarly, direct measurements of glomerular hemodynamic changes during infusion of antiglomerular basement membrane antibody [75] indicated a fall in K_f together with an increase in intraglomerular capillary pressure. On the other hand, a direct effect of TxA_2 on K_f is consistent with in vitro data showing that endoperoxide analogs contract rat isolated glomeruli as well as cultured mesangial cells, thereby presumably reducing the filtration surface area in vivo [6, 76].

Recent data have indicated an interesting strain difference between young SHR and age matched WKY in the renal vascular reactivity to locally administered angiotensin II and TxA_2 [77]. Unlike WKY, SHR display an exaggerated vascular reactivity to both angiotensin II and TxA_2, indicating that the degree of renal vasoconstriction in response to agonists may vary across strains of animals in a way that is genetically determined. Whether this would contribute to the sustained renal vasoconstriction of animals which are hypertensive remain to be established.

Angiotensin II, which induces TxA_2 synthesis by mesangial cells, also induces a concentration dependent increase in PGE_2 [52] and PGI_2 synthesis [78] that causes mesangial relaxation by promoting an increase in cyclic AMP. This modulating effect of PGE_2 and PGI_2 is lost in the presence of cyclooxygenase inhibitors, so that the mesangial response is amplified and GFR decreases. Similarly, whole glomeruli decrease their surface area when incubated with angiotensin II, and this effect is exacerbated by prostaglandin inhibition [76].

In a rat model of glycerol-induced acute renal failure the glomerular synthesis of PGE_2 decreased in the early phase of the disease while TxA_2 synthesis was greater in glycerol treated than in control rats only upon stimulation with arachidonic acid and at 24 hours after the glycerol administration [79]. These findings were consistent with other data showing that an inhibitor of TxA_2 synthesis partially protected another strain of rats against glycerol-induced acute renal failure [80]. Of interest, an angiotensin converting enzyme inhibitor did not confer protection in the same setting, but rather resulted in a significant increase in renal TxB_2 and 6-keto-$PGF_{1\alpha}$ synthesis. This may indicate that, at least in this model, TxA_2 is an important mediator of damage.

In a more physiological setting, the administration of cyclooxygenase inhibitors to conscious, sodium-replete animals does not induce changes in RPF and GFR, indicating that a compensatory role of vasodilatory prostaglandins for maintaining renal function is negligible under these circumstances [81–83]. By contrast, in anesthetized [84] or sodium-deprived animals [81],

or those with hemorrhagic hypotension [85] or congestive heart failure [86] blocking the cyclooxygenase leads to a fall in RPF and GFR. Thus, the potentially adverse effects of increased production of mesangial cell contractile agonists, including PGH_2/TxA_2, is partially offset by relaxant eicosanoids such as PGE_2 and PGI_2. Neither TxA_2, nor the other eicosanoids, appear to play an important role in mesangial function under physiological circumstances. Thus, it is not surprising that a thromboxane synthase inhibitor does not alter p-aminohippurate or inulin clearance in healthy humans [87], while a thromboxane receptor antagonist is capable of increasing RPF and GFR in patients with lupus nephritis [88].

Measurement of TxA_2 biosynthesis

Because of chemical instability of the bicyclic oxane structure, TxA_2 is rapidly hydrolyzed to the chemically stable and biologically inactive derivative, TxB_2. Thus, in vivo as well as in vitro measurements of TxA_2 biosynthesis have relied upon detection of TxB_2 or its enzymatic metabolites [89]. In healthy human beings, TxB_2 is produced at a very low rate, that is, 0.11 ng/kg min^{-1} [90], possibly reflecting the low frequency and/or intensity of stimuli to its secretion under physiologic circumstances. In contrast to the low rate of actual in vivo production, the capacity of platelets to synthesize TxA_2 ex vivo in response to appropriate stimuli (for example, thrombin) is quite substantial; 1 ml of human whole blood incubated at 37°C for one hour producing almost as much TxB_2 as the whole body during the same period of time, that is, roughly 300 to 400 ng [90]. TxB_2 undergoes two major pathways of metabolism in humans [91], one involving β-oxidation resulting in the formation of 2,3-dinor-TxB_2 and the other involving dehydrogenation of the hemiacetal alcohol group at C-11, resulting in the formation of a series of metabolites with a delta-lactone ring structure (such as 11-dehydro-TxB_2). These enzymatic changes are thought to occur primarily in the liver, lungs and kidney. A variety of techniques have been developed to quantitate the plasma levels and/or the urinary excretion of these metabolites [92, 93]. These include radioimmunoassay (RIA), enzyme-immunoassay (EIA) and negative ion-chemical ionization gas chromatography/mass spectrometry (NICI-GC/MS).

Largely indirect evidence supports the notion that the urinary excretion of primary unmetabolized eicosanoids, including TxB_2, reflects intrarenal eicosanoid production under physiological circumstances [89] in contrast to the metabolites which reflect mainly systemic, extrarenal production [89, 94]. The evidence concerning urinary TxB_2 derives from infusion studies of exogenous TxB_2 in healthy subjects [90], from the investigation of patients with lupus nephritis [88] and from pharmacologic studies of selective and nonselective cyclooxygenase inhibitors in health and disease [88, 95–97]. These human studies have measured the urinary excretion of TxB_2, and 2,3-dinor-TxB_2 and have demonstrated a dissociation of their excretory pattern (Table 1) in the setting of renal disease. No information of a similar nature is available from animal studies, even though measurements of urinary TxB_2 have been used extensively in rat and murine models of renal disease, as detailed below. Our recent finding that the rat kidney has the capacity to metabolize TxB_2 to 2,3-dinor-TxB_2 and that both are excreted in the urine, implies that under conditions of enhanced intrarenal synthesis of TxA_2, one should expect increased urinary excretion of both TxB_2 and 2,3-dinor-TxB_2 [98]. Correspondingly, while urinary TxB_2 may largely derive from the kidney under physiological circumstances, augmented extrarenal TxA_2 biosynthesis would be expected to be reflected by increased excretion of TxB_2 as well as by its metabolites. Thus, urinary TxB_2 (and its metabolites) is increased in patients with platelet activation [99] and following both inhalation and ingestion of TxB_2 [100]. Whether proteinuria alters the disposition of glomerular TxA_2 or its availability for further hydrolysis in urine remains to be determined.

Table 1. Differential pattern of excretion of thromboxane B_2 and 2,3-dinor-thromboxane B_2 in health and disease

Variable	Urinary excretion	
	Thromboxane B_2	2,3-Dinor-thromboxane B_2
Thromboxane B_2 infusion at 0.1 ng/kg · min^{-1}	no change	time-dependent increase
Aspirin *20–160 mg/day*	no change	dose-dependent decrease
Sulindac *200–800 mg/day*	no change	dose-dependent decrease
Lupus nephritis	2- to 5-fold increase	no change

Based on data from references 89–91 and 95–97.

Measurement of ex vivo glomerular TxB_2 production in response to added substrate or other stimuli represents a capacity-related index, conceptually similar to the measurement of TxB_2 production during whole blood clotting [101]. Measurement of TxB_2 synthesis in isolated glomeruli has been used extensively to monitor disease-associated changes in PGH_2/TxA_2 metabolism. However, the relationship of enhanced glomerular TxB_2 production, as detected ex vivo, to the actual rate of TxA_2 biosynthesis in vivo remains to be defined. Measurement of eicosanoid production in isolated glomeruli may provide useful information on qualitative differences in disease-related expression of PG-endoperoxide metabolism, possibly reflecting changes in the cellular source(s) of TxA_2 production or upregulation of Tx-synthase gene expression.

Inhibition of TxA_2 synthesis and action

Effects on glomerular permeability to macromolecules

Little information is available thus far as to whether the course of renal disease may be modified by preventing TxA_2 synthesis or action. However, several recent studies in animal models and limited clinical investigation suggests that indices of disease severity may be modified by this type of intervention.

Some of the most encouraging information has derived from studies of drug-induced nephrosis. Thus, treatment with a selective TxA_2 synthase inhibitor was shown to reduce urinary protein excretion without changes in GFR in rats with adriamycin (ADR) induced nephrosis [102]. Whether this reflected a direct effect of TxA_2 on glomerular permeability to proteins or was the result of changes in glomerular hemodynamics induced by exaggerated renal TxA_2 synthesis was not established by that study. That pharmacological inhibition of thromboxane synthase ameliorates proteinuria in experimental nephrosis has

been confirmed [103, 104] using structurally distinct thromboxane synthase inhibitors in a different model of drug-induced (aminonucleoside) nephrosis. Thromboxane synthase inhibitors also reduced [105] membrane attack complex (MAC)-induced proteinuria in the isolated perfused model of passive Heymann nephritis. In this model, proteinuria develops acutely, making it unlikely that compositional changes in the basement membrane underlie the observation. Rather, MAC-mediated cytotoxicity of epithelial cells may explain proteinuria. Similar to the results reported earlier in ADR nephrosis, a thromboxane-synthesis inhibitor diminished proteinuria in both cases; however, the effect of thromboxane synthesis inhibition on proteinuria was only partial. Whether this is due to the fact that these compounds do not fully suppress the synthesis of thromboxane or that factors other than TxA_2 may be involved in mediating the altered glomerular permeability to proteins is still a matter of speculation. At least one study in humans has documented that inhibiting thromboxane synthesis may reduce proteinuria and induce remission of the nephrotic syndrome when it is resistant to conventional therapy [106].

A recent study of rats made diabetic with streptozotocin also showed that a thromboxane synthase inhibitor given for six months reduced urinary TxB_2 excretion by 80% and abolished the rise in urinary albumin that occurs with time in diabetes [107]. The thromboxane inhibitor also induced an increase in the clearance of inulin in diabetic animals as compared with controls. A similar study has shown that the exacerbation of proteinuria in streptozotocin rats by a high protein diet is ameliorated by thromboxane synthase inhibition [108]. No changes in blood pressure or inulin clearance were detected, suggesting a mechanism unrelated to the vasoconstrictive properties of TxA_2.

The relationship between enhanced glomerular thromboxane formation and the development of renal disease has also been recently explored in normotensive rats of the Milan Strain (MNS) that spontaneously develop an age-related form of proteinuria and progressive glomerulosclerosis [109]. When these animals were given a thromboxane synthase inhibitor, decreased glomerular TxB_2 production and proteinuria were observed together with substantial preservation of RPF and GFR [110]. Blood pressure was unaltered by the treatment; however, the number of sclerotic glomeruli was reduced.

In nephritis induced by anti-GBM antibody, inhibitors of thromboxane synthase reduced proteinuria and partially prevented deterioration of renal function in both rat [111] and rabbit models [112]. Similar results were obtained by the use of a PAF receptor antagonist. Since the PAF receptor antagonist depressed the increment in thromboxane synthesis in immunologically injured glomeruli, it was suggested that its beneficial effect is mediated by suppression of excessive intrarenal synthesis of TxA_2 [112].

These biochemical and pharmacological findings appear to establish that exaggerated renal thromboxane synthesis may impair the selective properties of glomerular capillaries. However, the mechanism(s) for such an effect are less clear. TxA_2 may increase glomerular permeability to proteins by a direct, albeit still ill-defined, effect on glomerular barrier size-selective properties. Indirect evidence in support of this hypothesis is the observation that in ADR nephrotic rats, the increased glomerular production of TxA_2 [102] is associated with alteration of both size and charge-dependent permeability of the glomerular barrier, as indicated by the enhanced fractional clearance of both neutral and sulfate dextrans as compared to control animals [113, 114]. Since micropuncture studies in these animals have shown no changes in the determinants of single nephron GFR [115, 116], it is tempting to speculate that TxA_2 can directly alter the selective properties of the glomerular capillary barrier. This possibility is further supported by the finding that part of the effect of PAF on glomerular membrane permeability is mediated via the secondary release of TxA_2. Another possibility is that TxA_2 functions as an intracellular messenger for the induction of epithelial cell morphological changes that are associated with leakage of protein through the glomerular capillary wall.

Effects on K_f

In nephrotoxic serum nephritis induced by anti-GBM antibodies, glomerular TxB_2 synthesis is enhanced coincident with the reduction in GFR [111]. Blocking thromboxane synthesis by two different inhibitors induced a partial recovery of GFR, suggesting an effect of TxA_2 on factors that regulate glomerular filtration [111]. Since GFR decreased more than RPF, it is likely that the K_f is impaired by anti-GBM serum. The theoretical possibility that blocking thromboxane synthesis is beneficial in anti-GBM, because it prevents a TxA_2-mediated decrease in K_f, is consistent with the potent contractile effect of PGH_2/TxA_2 mimetics on mesangial cells. This is likely to decrease K_f by reducing the filtration surface area [117].

Another example that indicates a possible role of TxA_2 in decreasing the glomerular filtration surface area in renal disease is provided by the acute toxicity of cyclosporin A (CsA). Animals and humans given CsA show a reduction in GFR associated with a progressive rise in the renal synthesis of TxA_2 [118–121]. The administration of a TxA_2 synthesis inhibitor results in a significant decrease in the urinary excretion of TxB_2 and an increase in GFR in the rat [119]. Enhanced renal synthesis of TxA_2 in CsA-treated animals correlates with the reduction in GFR but not RPF. This is consistent with the possibility that TxA_2 reduces the K_f by reducing glomerular surface area.

Effects on afferent and efferent arterioles

Besides having an effect on K_f, possibly mediated by a contractile action on mesangial cells, there is evidence that excessive renal TxA_2 synthesis can influence the tone of afferent and efferent arterioles in renal disease. In a rat model of endotoxin-induced acute renal failure, the acute increase in renal vascular resistance and decrease in RPF, GFR and filtration fraction were prevented by a thromboxane synthesis inhibitor which also abolished the excessive renal synthesis of TxA_2 [122]. The simultaneous decrease in RPF and GFR and the effect of the inhibitor in such a model are more consistent with a TxA_2-mediated effect on afferent and efferent arterioles than on K_f.

Increased renal production of TxA_2 appears also to mediate the glomerular vasoconstriction that is associated with persistent ureteral obstruction [123]. Treatment with a thromboxane synthesis inhibitor reverses the vasoconstriction and improves renal function in this model [124]. Inflammatory cells generate excessive amounts of peptidoleukotrienes (LTs) in ex vivo

perfused hydronephrotic kidneys and both endogenous and exogenous LTC_4 stimulates TxA_2 synthesis in the hydronephrotic but not in the contralateral kidney [125]. Renal vasoconstriction is abolished by pretreatment with either a thromboxane synthase inhibitor or a leukotrene receptor antagonist [125]. Evidence that excessive renal TxA_2 synthesis mediates impaired renal function has also been obtained in autoimmune lupus mice by showing increased intrarenal production of TxB_2 in parallel deterioration of renal function, as well as by dietary manipulation of this pathway [126, 127]. Moreover, Spurney et al [127] have found that TxA_2 receptor blockade increases GFR in MRL-lpr mice.

Impairment of renal function in human lupus nephritis may also be reversed by thromboxane antagonists. Pierucci et al [88] reported that a TxA_2 receptor antagonist but not low-dose aspirin induced a similar increase in GFR and RPF in such patients, consistent with an effect of enhanced TxA_2 either on afferent and efferent arterioles or on mesangial cells. It has also been documented that the intense vasoconstriction which is associated with rejection of renal allografts is associated with increased renal TxA_2 synthesis [128]. Treatment with a thromboxane synthase inhibitor was associated with a decrease in urinary TxB_2 and an increase in RPF and GFR. Since cyclophosphamide also decreased urinary TxB_2, a likely source of the increment in TxB_2 in this model is from infiltrating inflammatory cells.

Effects on progression of glomerular damage

In the last ten years, several studies have addressed the progressive nature of renal disease after renal mass has been reduced surgically. The most attractive hypothesis is that the increased glomerular pressure and flow that develops in response to the initial loss of functioning nephrons causes glomerular injury and that this eventually leads to glomerulosclerosis [129, 130]. Rats with reduced renal mass also have increased urinary excretion of TxB_2 and chronic administration of a thromboxane synthase inhibitor decreases urinary protein excretion and limits glomerular damage [131]. The thromboxane inhibitor in this model also markedly inhibited 'in vitro' platelet aggregation, suggesting that intrarenal platelet activation and glomerular thrombosis may play a role in the progression of the disease after a critical number of nephrons has been reduced. However, the TxA_2 synthesis inhibitor also lowered blood pressure, leaving open the possibility of an effect related to this property of the compound. Indeed, neither low dose aspirin nor a thromboxane receptor antagonist—both of which failed to influence hemodynamics—retarded progressive renal disease in the remnant kidney model [132, 133].

In another model of progressive renal insufficiency that shares many biologic similarities with the human condition, immune complex nephritis induced by ferritin-anti-ferritin complexes in Dahl-salt sensitive rats, a thromboxane synthase inhibitor, ameliorated urinary protein excretion and renal function [134]. Histological examination of the kidney showed that animals given the thromboxane synthase inhibitor were also significantly protected from glomerulosclerosis. These findings are consistent with those of others [110, 126] in other experimental models of progressive nephropathy (that is, Milan normotensive rats and NZB/NZW F_1 mice) in which a protective effect of thromboxane synthase inhibitors on renal injury was evident.

Manipulating the thromboxane biosynthetic pathway in renal disease may also be beneficial because it limits platelet activation, one of the possible mechanisms involved in the progression of renal disease. This possibility is consistent with an observational study that suggested that antiplatelet agents slowed the deterioration of renal function and prevented the development of end-stage renal disease in patients with membranoproliferative glomerulonephritis [135]. Intraglomerular platelet aggregation could facilitate mesangial cell proliferation and excessive generation of mesangial matrix. Thus, platelet-derived growth factor (PDGF) binds to specific receptors on cultured mesangial cells and stimulates DNA synthesis and cellular proliferation [136]. In vitro studies have documented that another platelet product, transforming growth factor-β (TGF-β) stimulates synthesis of the extracellular matrix components, collagen IV and laminin and the proteoglycans, biglycan and ecorin, by cultured mesangial cells [137, 138] without stimulation of cell proliferation. TGF-β mediates proteoglycan production and mesangial matrix expansion in vivo in a rat model of glomerulonephritis induced by anti-thymocyte serum [139].

An alternative possibility to explain why reducing renal TxA_2 synthesis is protective against glomerulosclerosis emerged from studies showing that PGH_2/TxA_2 analogs induce a dose-dependent increase in laminin A, B_1 and B_2 chains in tumor cells in culture and that these effects are reversed by a thromboxane receptor antagonist [140]. This possibility is further supported by the recent observation that a thromboxane synthase inhibitor ameliorated progressive diabetic nephropathy and prevented the increase in type IV collagen mRNA levels in mice with non-insulin dependent diabetes mellitus [141].

Implications of pharmacologic studies

PGH_2 and TxA_2 exert multiple actions within the kidney which may contribute to the progression of renal disease. These include constriction of glomerular afferent and efferent arterioles, intraglomerular platelet aggregation, contraction of glomerular mesangial cells and proliferation of mesangial cell matrix. These effects have been characterized using stable PGH_2/TxA_2 analogs and although radioligand studies suggest a single class of specific binding sites in glomeruli, it is unclear whether these are subtypes distinct from those in platelets and vascular smooth muscle cells and whether their affinity for PGH_2 and TxA_2 is similar. In this regard, much of the information suggesting the functional importance of this system has been obtained with specific inhibitors of thromboxane synthase, an intervention that might be expected to lead to accumulation of PGH_2, coincidental with inhibition of TxA_2 synthesis.

Such studies have implicated TxA_2 in the nephrotoxicity of pharmacologic, immune and toxic insults, in the progression of chronic renal disease and in renal allograft rejection. Biochemical assessment of renal biosynthesis of TxA_2 has facilitated the identification of potential targets for pharmacological intervention. This evidence has been accumulated particularly in animal models, but also, to a limited extent, in humans. It suggests that pharmacological inhibition of TxA_2 synthesis or action merits

Table 2. Thromboxane A_2 in experimental and human renal diseases

Disease model	Site of abnormal TxA_2 synthesis	Changes in urinary excretion of TxA_2 metabolites	Effect of pharmacological inhibition of TxA_2 synthase/receptor
Experimental			
Endotoxin-induced ARF (rat)	cortex [118]		
Nephrotoxic nephritis (rat)	glomeruli [74]		↓ proteinuria, ↑ GFR and RPF [74]
Glycerol-induced ARF (rat)	glomeruli [79]		↑ GFR [80]
Adriamycin nephrosis (rat)	glomeruli [102]	↑ TxB_2 [102]	↓ proteinuria [102]
Puromycin nephrosis (rat)			↓ proteinuria [103]
Passive Heymann nephritis (rat)			↓ proteinuria [105]
STZ-induced diabetes (rat)		↑ TxB_2 [107]	↓ albuminuria, ↑ GFR [107]
Milan normotensive strain (rat)	glomeruli [119]		↓ proteinuria [110]
Nephrotoxic nephritis (rabbit)	glomeruli [112]		↓ proteinuria, ↑ GFR [112]
CsA nephrotoxicity (rat)	glomeruli [121]	↑ TxB_2 [119, 120, 121] ↑ 2,3-dinor-TxB_2 [120, 121]	↑ GFR [119]
Endotoxin-induced ARF (rat)	cortex [122]		↑ GFR and RPF [122]
Ureteral obstruction (rat, rabbit)	kidney microsomes [123]		↑ GFR and RBF [124]
Renal allograft rejection (rat)			↑ GFR and RPF [128]
Renal mass ablation (rat)		↑ TxB_2 [131]	↓ proteinuria, ↑ GFR and RPF, ↓ FSG [131]
Dahl-S strain (rat)	glomeruli [134]		↓ proteinuria, ↑ GFR, ↓ FSG [134]
Noninsulin-dependent diabetes (mouse)			↓ cortex type IV collagen mRNA [141]
Lupus nephritis (mouse)	cortex [126, 127] medulla [126]		↓ GFR [127]
Clinical			
Lupus nephritis		↑ TxB_2 [88]	↑ GFR and RPF [88]
Nephrotic syndrome		↑ 2,3-dinor-TxB_2 [106]	↓ proteinuria [106]
CsA nephrotoxicity		↑ 2,3-dinor-TxB_2 [118]	

continued evaluation in the treatment of a variety of renal diseases in humans.

Because PGH_2 shares with TxA_2 many of the renal actions that have been discussed in this review, it is puzzling that pharmacological blockade of thromboxane synthase, which results in accumulation of PGH_2 in platelets [142, 143], was shown so consistently effective in ameliorating TxA_2-dependent loss of renal function and structure. This is at variance with the rather disappointing results obtained with thromboxane-synthase inhibitors when used as potential antithrombotic agents [144]. A number of studies both in vitro [145] and in vivo [146, 147] have demonstrated that PG endoperoxides modulate the functional response of platelets to thromboxane synthase inhibition and may, in fact, substitute for the proaggregatory activity of TxA_2.

Several factors might contribute to this apparent discrepancy. These include: (a) a different biosynthetic capacity of platelets, vis-à-vis glomerular cells, in producing TxA_2, resulting in different levels of "critical" pharmacological suppression; (b) a lower affinity of PGH_2 than TxA_2 for the putatively shared glomerular mesangial and arteriolar receptor(s); (c) a markedly different metabolic fate of PGH_2 in platelets (largely converted to TxA_2 by thromboxane synthase) versus glomerular mesangial and epithelial cells (perhaps largely converted to PGE_2 and PGI_2 by other isomerases) resulting in much greater accumulation of PGH_2 in platelets than in glomerular cells, in the face of comparable blockade of thromboxane synthase. Whether PGH_2/TxA_2-receptor antagonists may mimic or enhance the renal effects of thromboxane synthase inhibitors largely remains to be investigated and may shed some light on this controversial issue.

Summary and conclusions

PGH_2 and TxA_2 exert their actions via tissue specific, receptor isoforms. PGH_2/TxA_2-dependent platelet aggregation and contraction of vascular and bronchial smooth muscle and of glomerular mesangial cells occur via receptors linked to activation of phospholipase C.

Although PGH_2/TxA_2 appear to be of little importance in the maintenance of renal function under physiological circumstances, increased renal TxA_2 biosynthesis has been documented in a variety of animal models of renal disease and in some clinical disorders (Table 2). The effects of this eicosanoid on renal tissues in vitro and of pharmacological manipulation of TxA_2 synthesis and action in vivo suggest that such interventions will provide new drugs for the treatment of human kidney disease.

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